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BAZI YENİ SUBSTİTÜE α , N–DİARİLNİTRONLAR ÜZERİNDE SENTEZ, KARAKTERİZASYON VE KROMATOGRAFİK ÇALIŞMALAR

SYNTHESIS, CHARACTERISATION AND CHROMATOGRAPHIC STUDIES ON SOME NEW SUBSTITUTED α, N–DIARYLNITRONES

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SUMMARY

The one step, simple and rapid oxidation procedure by metachlorperbenzoic acid on certain ring substituted secondary anilines was employed to prepare nitrone functional groups required as potential metabolites and/or intermediates for drug metabolism studies. The structures and purity of purity of nitrones were confirmed using spectroscopic methods and their separations from both starting products and corresponding primary anilines were achieved by use of thin–layer and high performance liquid chromatographic techniques.

ÖZET

Halkada substitue olmuş çeşitli sekonder anilinlerin metaklorperbenzoik asidle tek kademeli basit ve hızlı oksidasyon reaksiyonu ilaç metabolizma çalışmalarının metabolitleri ve ara ürünleri olarak gereksinilen nitron fonksiyonel gruplarının sentezi için uygulandı. Nitron yapıları ve saflığı spektroskopik metodlarla aydınlatıldı. Nitronların hem başlangıç maddelerinden, hemde karşılık olan primer anilinlerden ayrılmaları ince tabaka ve yüksek basınçlı sıvı kromatografik tekniklerle sağlandı.

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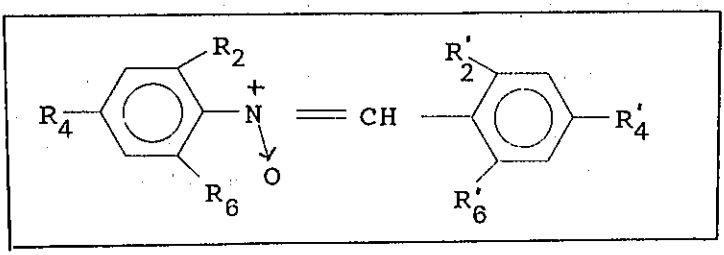
Table – 1 : List of Abbreviations

Aniline	A
Benzaldehyde	B
2, 4, 6–trimethylaniline	TMA
N–benzyl–2, 4, 6–trimethylaniline	NBTMA
α–phenyl–N–(2, 4, 6–trimethylphenyl) nitrone	PTMPN
2, 4, 6–trichloroaniline	TCA
N–benzyl– 2, 4, 6–trichloraniline	NBTCA
α– phenyl–N–(2, 4, 6–tirchlorophenyl) nitrone	PTCPN
N–(2,4–dichlorobenzyl) aniline	N24DCBA
α–(2,4–dichlorophenyl)–N–phenylnitrone	24DCPPN
2,4–dichlorobenzaldehyde	24DCB
N–(2,6–dichlorobenzyl) aniline	N26DCBA
α–(2,6,–dichlorophenyl)–N–phenylnitrone	26DCPPN
2,6–dichlorobenzaldehyde	26DCB
metachlorperbenzoicacid	3CPBA

INTRODUCTION

The diaromatic nitrone function (figure 1) is an important product in the metabolism of certain secondary amines (1, 2).



Nitrone	R_2	R_4	R_6	R_2'	R_4'	R_6'
PTCPN	Cl	Cl	Cl	–	–	–
PTMPN	CH_3	CH_3	CH_3	–	–	–
24DCPPN	–	–	–	Cl	Cl	–
26DCPPN	–	–	–	Cl	–	Cl

Figure – 1 : Structures of substituted α, N–diaryl nitrones

In order to establish their possible role as metabolic intermediates in the microsomal metabolism of ring substituted N–benzylanilines, the corresponding nitrone function for each secondary aniline was required to be used as an authentic standard. A review of the preparation of this class of compounds has previously been reported (3). The condensation of

appropriate aldehyde and phenylhydroxylamine (4) has been used to prepare nitrones. However, this is a two step synthesis and extreme care must be taken due to tha facile decomposition of the hydroxylamine. The preparation of nitrones from parent amines by direct oxidation with metachlorperbenzoic acid has been used for preparing aromatic–aliphatic (5) and diaromatic nitrones (3) as a convinient one step method.

MATERIALS AND METHODS

Materials

The parent secondary anilines were synthesised as reported elsewhere (6) by the modification of Vogel's classical benzylation procedure (7) and their purity was confirmed using spectrometric and chromatographic techniques. Primary anilines, corresponding aldehydes and metachlorperbenzoic acid were purchased from Aldrich Chemical Company, UK. Thin–layer chromatographic solvents (S. L. R. grade) and acetonitrile (HPLC grade) were obtained from British Drug Houses, Dorset, UK. Plastic–backed TLC plates precoated with silicagel 60 F_{254} were obtained from Merck Company, Darmstad, Germany.

Instruments

Mass spectra were determined by direct insertion of samples in methanol on a VG12F mass spectrometer with a 35 ev ionisation potential, source temperature 200–240 °C. NMR Spectra were determined on a Perkin Elmer R32 90 Mz instrument. Deuterated chloroform and TMS were used as sample solvent and internal standard respectively. UV Spectra were obtained on a Kontron–Uvikon 860 UV spectrophotometer, the samples were prepared as 10^{-3} molar solutions in methanol. C, H, N analyses were carried out on model 240XY Control and 1106 Carlo Erba equipent. The HPLC column (Spherisorb μ–Bondapak C18 5 μm (25 cm length X 4.6 mm internal diameter) was purchased from Phase Separations Limited, Deeside, UK.

High Performance Liquid Chromatograph

The HPLC chromatograph consisted of an isocratic system comprising one LCD Analytical contaMetric 3200 solvent delivery system, a Rheodyne syringe loading sample injector valve (model 7125) fitted with a 20 μl sample loop, a Milton ROY spectroMonitor–3100 Variable wavelength UV detector, and a Milton ROY model 4000

Computing Integrator. Phosphate buffer (pH : 7.4, 0.02 M) was used to regulate the pH of the mobile phase. Acetonitrile and buffer were filtered throught a sartorius 40 μm filter (Sartorius Instruments Ldt., Belmont, Surrey), degassed by a gentle stream of helium gas for 10 minutes followed by sonication for 5 minute prior to use. This was carried out in order to prevent the formation of air bubbles and large particles (> 40 μm) from entering the HPLC system.

Method

Synthesis of substituted α, N–diphenylnitrones : To a cooled (0–5 °) solution of the N–benzyl–4–substituted aniline (0.01 mol) in 25 ml dry acetone kept in the dark was added dropwise a solution of 3–CPBA (0.02 mol, 3.5 g) in 25 ml dry acetone over lh. The reaction was terminated by removal of the solvent under reduced pressure to leave an orange/yellow solid. The solid was dissolved in 50 ml diethylether containing a small quantity of dichloromethane. The solution was washed with aqueous potassium carbonate (0.5 M, 3X25 ml), distilled water (25 ml), dried (anhydrous $MgSO_4$) and concentrated to leave a solid. Trituration of the solid with ice cold diethylether afforted crytals of substituted α, N–diphenylnitrone. The product, recrystallised from hot diethylether was obtained in yields from 40–65 %. In all cases TLC and HPLC examination showed a single product.

PTMPN recovered as pale yellow crystals, m.p. 92°. IR : 1170 cm^{-1}. UV (methanol) λ_{max} = 297 nm. M.S. : m/e = 239(58), 223(74), 222(100), 135(45), 105(11), 77(12) . ^1H–NMR ($CDCl_3$) : (ppm) = 2.30(s, 9 H, methyl protons), 6.92(s,2H, meta to the N–phenyl ring), 7.4–7.6 (m, 4H, CH and meta & para protons on α – phenyl ring), 8.3–8.5 (m, 2H, ortho on α–phenyl). (Found : C,79.38; H, 7.12; N, 5.75. Requires : $C_{16}H_{17}NO$: C, 80.30; H, 7.16; N, 5.85).

PTCPN recovered as white needles, m.p.119° . IR : 1170 cm^{-1} . UV (methanol) λ_{max} = 309 nm. M.S. : m/e = 211(17), 195(31), 105(100), 77(3). (Found : C,51.85; H,2.70;N,4.5. Requires : $C_{13}H_8Cl_3NO$: c, 51.95;H,2.68; N, 4.66).

24DCPPN recovered as white crystals, m.p. 88°. IR : 1170 cm^{-1}. UV (methanol) λ_{max} = 206,241 and 321 nm. M.S. : m/e = 269(1), 266(4), 265(10), 250(2), 232(17), 231(8), 230(51), 91(100), 77(14). 250(2). (Found : C, 58.61 ; H, 3.35; N,5,02. Requires : $C_{13}H_9C_{12}NO$: C,58.67; H,3.41; N,5.26).

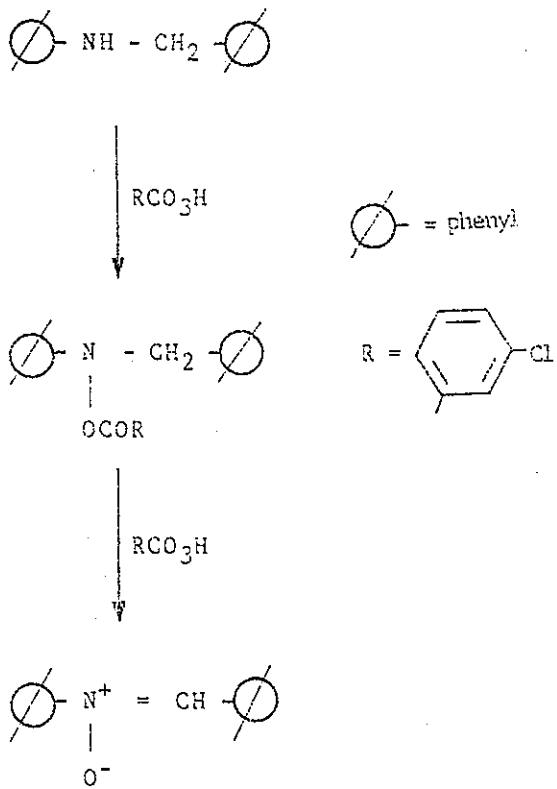


Figure – 2 : Reation equation for the formation of nitrone function from substituted N–benzylanilines via an ester by 3CPBA

26DCPPN recovered as white crystals, m.p.104 °. IR : 1170 cm^{-1}. UV (methanol) λ_{max} = 204 and 276 nm. M.S. : m/e = 269 (1), 266(1), 250 (2), 232(34), 231(15), 230(100), 91(38), 77(29). ^{1}H–NMR ($CDCl_3$) : (ppm) = 7.3–7.6 (m, 6H, meta and para hydrogens on both rings), 7.7–7.9 (m, 2H, ortho hydrogens on N–phenyl ring), 8.08 (s,1H, CH = N). (Found : C,58.72; H,3.40; N, 5.39. Requires : $C_{13}H_9Cl_2NO$: C, 58.67 ; H, 3.41; N, 5.26).

Preparation of HPLC and TLC conditions : Various compositions of acetonitrile and buffer solution were tried in order to achieve good separation of nitrones from secondary anilines, primary anilines and corresponding aldehydes using the HPLC system described above. Commercially available precoated plastic plates and several solvent combinations were also employed to achieve TLC separation of nitrones

and other potential metabolites. The Rf values of compounds visualised under UV light and compounds responding to chromogenic reagents recorded. The solvent systems used were S1, petroleumether (b.p. 40 – 60 °C) – acetone (70:30 v/v); S2, benzene–ethylacetate (90:10, v/v); S3, petroleumether (b.p. 40–60 °C) – ethylacetate (90:10, v/v). The chromogenic reagents D1 Ultraviolet light 254 nm, D2 Ammonical silver nitrate (Tollen's reagent); silver nitrate (10 %) treated with sodium hydroxide (2M) until precipitation complete. Mixture treated with ammonia (sp. gr. 0.88) until a clear solution obtained, D3 Ferric chloride/ bathophenanthroline; methanolic solutions of iron(III) chloride (0.005 %) and 0.1 % bathophenanthroline (4,7–diphenyl–1,10–phenanthroline) mixed in equal volumes, immediately before use, D4 Sodium aminoprusside (SAP); 0.05 % in 20 % aqueous ethanol containing 1 % magnesium chloride, D5 Alkaline tetrazolium reagent; 0.1 % 2,3,5–triphenyltetrazolium chloride in ethanolic sodium hydroxyde (0.5 M) prepared fresh, D8 Ehrlich reagent; 10 % p–dimethylaminobenzaldehyde in conc. HCl mixed with acetone 1:4 just before use.

RESULTS AND DISCUSSION

The reaction of 3CPBA on substituted N–benzylanilines gave rise to corresponding nitrones in a pure state. The stability of starting materials (N–benzylanilines) gave considerable advantage over the alternative synthesis employing phenylhydroxylamine and the corresponding aldehyde where the reactive nature of the hydroxylamine demands it being freshly prepared prior to use. Analytical data presented in the experimentel section confirmed the purity and authenticity of the compuounds synthesised. NMR analysis confirmed the structures. Chemical shifts are as expected on the basis of the findings of Gorrod and Gooderham (3). The IR spectrum showed a band at 1170 cm^{-1} consistent with the literature values for N–O strectching bonds characteristic of nitrones (3, 9). The UV spectra of nitrones exhibited bathochromic shifts to about 276 to 321 nm upon conjugation with the aromatic ring (9, 10). The mass spectra of substituted α, N–diphenylnitrones have been studied by several groups. In all spectra a M–16 peak is present which results from the loss of oxygen together with molecular ion peaks (11). The fragmentation patterns from the present work are consistent with the literature data for other diarylnitrones (11).

Thin layer chromatography and response to chromogenic reagents of some diaryl nitrones has been previously studied by Gorrod and Gooderham (8) although this data does not include TLC separation of the compounds of present interest. The chromatographic properties of nitrones together with their corresponding amines and colour responses, to chromogenic reagents are shown in Table 2. The solvent systems recorded were found to be the best overall systems for the separation of the compounds used. Tollen's reagent reacted slowly with the nitrones. Nitrones also gave colours with D2, D3, D4 and D5 detection systems. This is consistent with the observation by Gooderham and Gorrod (8). Ehrlich reagent immediately gave a yellow colour with all the compounds tested.

Table – 2 : Thin layer chromatographic separation and detection of nitrones and starting material.

Compound	Rf x 100 value			Chromogenic response				
	S1	S2	S3	D1	D2	D3	D4	D5
TCA	66	85	–	+	–	–	–	–
NBTCA	72	91	–	+	–	–	–	–
PTCPN	56	70	–	+	Blue	Red	Pink	Red
TMA	55	30	–	+	–	–	–	–
NBTMA	74	55	–	+	–	–	–	–
PTMPN	49	14	–	+	Blue	Red	Pink	Red
A	–	40	12	+	–	–	–	–
N24DCBA	81	83	44	+	–	–	–	–
24DCPPN	71	53	28	+	Blue	Red	Pink	Red
N26DCBA	41	80	52	–	–	–	–	–
26DCPPN	26	24	8	+	Blue	Red	Pink	Red

(for solvents (S) and detection systems (D) see text)

Various proportions of acetonitrile : buffer and various flow rates were examined for the separation of compounds under study. The systems with the mobile phase composition described in Table 3 were found to give the best separations. In all cases, there was no difficulties

Table – 3 : HPLC systems used for the separation of nitrones from other potential metabolites of secondary anilines :

System	Mobile phase composition (v/v) (Acetonitrile : 0.02M phosphate buffer)	pH	Flow rate (ml/min)
S1	(45:55)	7	2
S2	(65:35)	4	1
S3	(40:60)	7	2

(pH adjusted with glacial acetic acid)

in the separation of nitrones and corresponding anilines. The benzylic anilines, as they are very liphophilic compounds, had a longer retation time than nitrones and primary anilines on the reverse phase HPLC system (Table 4). In order to achieve tha separation of these compounds,

Table – 4 : HPLC retention times of nitrones together with corresponding primary, secondary anilines and aldehydes :

Compound**	Retention time (min)		
	S1*	S2	S3
B	3.3	4.2	ND
TMA	5.5	ND	ND
NBTMA	29.9	ND	ND
PTMPN	8.2	ND	ND
TCA	ND	7.3	ND
NBTCA	ND	15.2	ND
PTCPN	ND	5.3	ND
A	ND	ND	2.5
N24DCBA	ND	ND	41.6
24DCPPN	ND	ND	19.4
N26DCBA	ND	ND	31,1
26DCPPN	ND	ND	5.9
24DCB	ND	ND	8.1
24DCPPN	ND	ND	6.3

* for solvent systems see table 3
** for abbreviations see table 1
ND : not determined

several acetonitrile: buffer compositions with different flow rates were tried. The best separation of the compounds of interest under these conditions are shown in Table 4. A gradient HPLC system has previously been used for the separation of certain N–benzylanilines from their metabolites (8). The use of a gradient system has a number of disadvantages ie baseline drifting within the run and the requirement for a rather long re–equilibration time between each run which increases analysis time. Therefore, the isocratic mode of HPLC described proved useful in the separation of nitrones from substituted N–benzylanilines, the corresponding primary anilines and aldehydes.

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